Neurosci Bull December 1, 2013, 29(6): 737–744. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1378-z

·Original Article·

Antidepressant-like effects and memory enhancement of a herbal formula in mice exposed to chronic mild stress

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ABSTRACT

Shen Yuan Gan (SYG) is a Chinese herbal prescription composed of total saponins of *Panax ginseng* and total oligosaccharide esters of *Polygala tenuifolia* (2:1). Our previous studies have demonstrated that SYG has antidepressant-like effects in various mouse models of behavioral depression. The present study aimed to test whether SYG affected chronic mild stress (CMS)-induced depression and cognitive impairment in mice. We found that a 5-week CMS schedule induced significant degradation of the coat state, decreased sucrose intake in the sucrose-preference test, and increased the latency to feed in the novelty-suppressed feeding test. All of these CMS-induced changes were ameliorated by SYG (100 and 200 mg/kg) and fluoxetine (10 mg/kg). In addition, SYG restored the decreased monoamine neurotransmitter concentrations (serotonin, dopamine, norepinephrine and acetylcholine) induced by CMS in the prefrontal cortex. Interestingly, SYG ameliorated CMS-induced cognitive impairment in the step-through test, and increased the acetylcholine level in the prefrontal cortex. These results suggest that SYG has an antidepressant-like action and enhances cognition by modulating the serotonin, dopamine, norepinephrine, and acetylcholine levels in the prefrontal cortex.

Keywords: mice; antidepressant; neurotransmitter; memory; *Panax ginseng*; *Polygala tenuifolia*

INTRODUCTION

Depression is a common psychiatric disorder with significant potential morbidity, mortality, and disability. It is considered a significant risk factor for cardiovascular disease[1] and a multitude of neurological disorders, including dementia[2]. However, the current pharmacotherapy remains limited. Many antidepressants have undesirable side-effects, including sleep disturbance[3], sexual dysfunction[4] and cognitive impairment[5]. Thus, there is a great need for safer, better-tolerated, and more effective treatments for depression.

Recent studies have shown that herbal medicines may provide prospective alternatives in the treatment of depression due to their safety profile and tolerability[6-8]. *Panax ginseng* and *Polygala tenuifolia* are components of the most popular traditional oriental medicines. The two herbs are often combined in formulas (such as Kanxin-San and Dingzhixiao-wan) prescribed for depressive-like disorders[9]. Total saponins of *P. ginseng* (GTS) and total oligosaccharide esters of *P. tenuifolia* (PTG) are known to be the antidepressant ingredients[10,11]. In a previous study, we combined GTS and PTG to form the Shen Yuan Gan (SYG) prescription. Using factorial design analysis of variance, we found that the SYG prescription has better antidepressant effects than either alone in the tail suspension and forced swimming tests[12]. In the present study, we further investigated the antidepressant-like effects of SYG in a chronic mild stress (CMS) model and the underlying mechanisms, to explore its potential use as

an antidepressant. The cognitive effect of SYG was also assessed.

MATERIALS AND METHODS

Preparation of SYG

Dried roots of *P. ginseng* (1 kg) and *P. tenuifolia* (1 kg) were separately extracted three times with boiling water (1 h each), then the filtrates were placed on columns containing D101 macroporous resin (2 kg). The column with *P. ginseng* was eluted successively with water and 80% ethanol, and the 80% ethanol elution was evaporated to obtain a saponin-enriched fraction (80 g). The *P. tenuifolia* column was eluted to colorless or pale liquid with 10% ethanol, then with 60% ethanol thoroughly, and the 60% ethanol was concentrated to obtain an oligosaccharide ester-enriched fraction (50 g). SYG was made as a 2:1 mixture of the above extracts. Fluoxetine was obtained from the National Institutes for Food and Drug Control (Beijing, China). All drugs were dissolved in distilled water.

Animals

BALB/c mice (26–28 g) were purchased from the Laboratory Animal Institute of the Chinese Academy of Medical Science Center (Beijing, China). The animals were housed in polypropylene cages under standard experimental conditions (20–22°C, 55% humidity, food and water *ad libitum*, 12:12 h light/dark cycle). Animals were acclimatized for one week before the experiments. All experiments were performed in compliance with the guidelines of the Principles of Laboratory Animal Care (NIH publication No. 80-23, revised 1996) and approved by the ethical committee for the use of experimental animals of the Institute of Medicinal Plant Development (China).

CMS Model

Sixty male mice were used in the CMS experiment. Before the CMS protocol, the baseline sucrose preference of the animals was measured and then they were assigned to five groups (12/group): control, CMS, CMS + 10 mg/kg fluoxetine, CMS + 100 mg/kg SYG, and CMS + 200 mg/kg SYG. During the CMS experiment, the mice in the control group were left undisturbed in cages in a separate room, whereas mice in the other four groups were separately housed in individual cages and exposed to CMS. SYG or fluoxetine was administered orally once daily from the beginning of the stress regime. Mice in the control and CMS groups were given distilled water daily (Fig. 1).

CMS was induced as described previously[13], with slight modifications. Briefly, mice were submitted daily to 2–3 different stressors for five weeks in a chronic and unpredictable way. The stressors were sawdust deprivation, damp sawdust, soiled cage (aversive odor due to old rat sawdust), social stress (switching cages), predator sounds for 30 min (cat, dog), inversion of the light/dark cycle, and food or water deprivation for 12 h. The animals were subjected to these stressors randomly at any time of the night or day.

Coat State and Body Weight

Coat state was assessed before and after five weeks of CMS. Eight regions were evaluated: head, neck, dorsal, ventral and genital areas, forepaws, hindpaws and the tail. A score of 0 indicated good coat condition, and 1 indicated a dirty coat. The final score for the coat state was calculated as the sum of scores divided by the total number of regions[14]. Body weight was recorded every week.

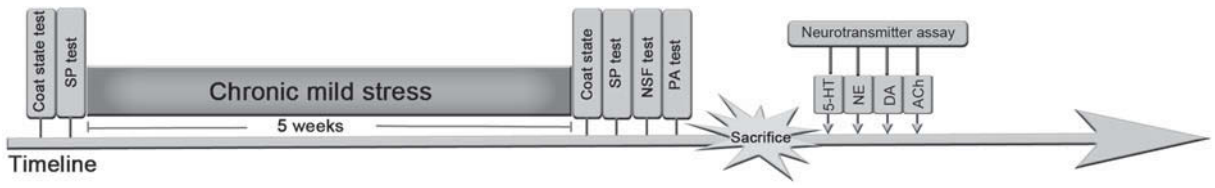

Fig. 1. Schematic of the experimental design. Baseline sucrose-preference and coat state were assessed before beginning the chronic mild stress (CMS) regimen. The regimen lasted 5 weeks. SYG or fluoxetine was administered daily by gavage during both the 5-week stress sessions and behavior tests. After the regimen, behavioral tests were carried out: coat state, sucrose-preference (SP) test, novelty-suppressed feeding (NSF) test, and passive avoidance (PA) test. Following the PA test, the mice were decapitated and then sacrificed for neurotransmitter assay. 5-HT, 5-hydroxytryptamine; ACh, acetylcholine; DA, dopamine; NE, norepinephrine.

Sucrose-preference Test

The sucrose-preference test was carried out before and at the end of the 5-week CMS exposure. The test was performed as described previously, with minor modifications[15]. Mice were first trained to experience and drink sucrose solution (1%) for 24 h, by presenting them simultaneously with two identical bottles, one with sucrose solution (1%) and the other tap water. In the testing protocol, mice were given a free choice between the two bottles for 15 h. The position of the bottles was switched at the mid-point of testing to prevent possible effects of side preference in drinking behavior. The preference for sucrose was calculated as: sucrose preference = sucrose intake (g) / [sucrose intake (g) + water intake (g)] ×100%.

Novelty-suppressed Feeding Test

The novelty-suppressed feeding test was similar to the version used by Vollenweider[16]. After 24 h of food deprivation, a single food pellet (regular chow) was placed in the center of the box (42 × 31 × 20 cm^3). The animal was placed in a corner of the box and the latency to bite was recorded. Immediately afterwards, the mouse was returned to its home cage and the amount of food consumed during the subsequent 5 min was measured (home food-consumption).

Step-through Passive Avoidance Test

The step-through passive avoidance apparatus and procedure were as described earlier[17] with minor modifications. Briefly, mice were first habituated to the light and dark chambers for 5 min (training trial). In the acquisition trial, the mice were placed in the dark chamber opposite the guillotine door and an inescapable foot-shock (0.8 mA) was delivered through the grid floor. After 24 h, the mice were placed in the light chamber for the retention trial. The number of times the mice stepped into the dark chamber and the time spent in the dark chamber within 5 min were recorded by software developed by our own institute.

Measurement of Neurotransmitter Levels in the Prefrontal Cortex

The concentrations of 5-hydroxytryptamine (5-HT), norepinephrine (NE), dopamine (DA) and acetylcholine (ACh) in the mouse prefrontal cortex were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). After the passive avoidance test, the mice were sacrificed by decapitation, the brain was rapidly removed on ice, and the prefrontal cortex dissected out. The tissues were weighed, homogenized and mixed with 0.2% formic acid in acetonitrile. After centrifugation at 12 000 rpm for 10 min at 4°C, an aliquot (200 μL) of the supernatant was collected and mixed with 20 μL internal standard solution (300 μg/mL, 3,4-dihydroxybenzylamine, DHBA); then 50 μL of the mixture was injected into the LC-MS/MS system for assay. The neurotransmitters and internal standards were detected in multiple reaction monitoring mode, at m/z 177.2→160.0 (5-HT), 170.3→152.2 (NE), 154.2→137.1 (DA), 146.1→87.1 (ACh) and 140.1→123.1 (DHBA as internal standard). The peak area ratios of analyte *versus* internal standard were used to quantify the neurotransmitter concentrations.

Statistical Analyses

Data are expressed as mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by LSD *post hoc* tests for inter-group comparisons. Significant difference was determined at P <0.05.

RESULTS

Effect of SYG on the Degradation of Coat State and Body Weight in Mice Exposed to CMS

There was no significant difference in the coat state among the groups before CMS exposure (data not shown), but it was degraded after 5 weeks of CMS (Fig. 2). CMS significantly increased the coat state index compared to the control group, while fluoxetine (10 mg/kg) and SYG (100 and 200 mg/kg) all counteracted this effect.

A significant reduction in body weight in the CMS group was found at the end of the fifth week. Neither fluoxetine nor SYG had any effect on the change in body weight, compared with the CMS group (Table 1).

Effect of SYG on Sucrose Consumption in Mice Exposed to CMS

Before CMS exposure, there was no significant difference in the sucrose consumption among groups (Fig. 3A). After 5 weeks of exposure to stress, this was significantly reduced in the CMS mice compared with the control mice. SYG (100 and 200 mg/kg) and fluoxetine (10 mg/kg)

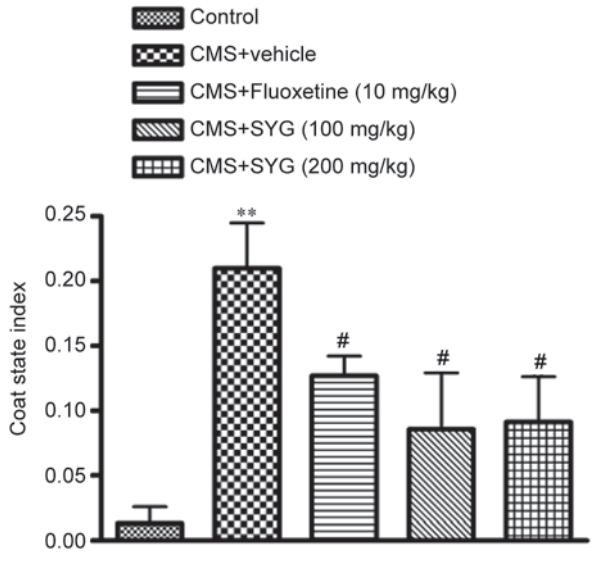

Fig. 2. Effect of SYG on coat state index in control and CMS mice. Values are mean ± SEM (*n* = 12). ***P* <0.01 compared with control group, #*P* <0.05 compared with CMS group.

showed significant improvements in sucrose consumption compared with the CMS group.

Effect of SYG on the Latency to Feed in Mice Exposed to CMS

The CMS group exhibited a longer latency to feed in the novelty-suppressed feeding test than the control group (Fig. 4). Chronic treatment with SYG (100 and 200 mg/kg) and fluoxetine (10 mg/kg) significantly reduced the latency compared with the CMS group. SYG and fluoxetine did not alter food intake in this test (*P* >0.05, data not shown).

Effect of SYG on Cognitive Deficit in the Step-through Test in Mice Exposed to CMS

The number of errors (Fig. 5A) and the time in the dark chamber (Fig. 5B) were significantly increased in the CMS group compared with the control group. SYG (100 and 200 mg/kg) significantly decreased the number of errors and the time in the dark chamber compared to the CMS group. But

Table 1. Effect of SYG on body weight (g) in mice (*n* = 12)

Groups	Dose (mg/kg)	Body weight (g)					
		Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
Control	-	27.65 ± 0.41	27.90 ± 0.39	27.85 ± 0.41	28.31 ± 0.48	29.00 ± 0.61	30.36 ± 0.67
CMS	-	27.20 ± 0.39	26.75 ± 0.50	28.31 ± 0.44	28.42 ± 0.38	27.98 ± 0.55	27.28 ± 0.3**
CMS+Fluoxetine	10	28.45 ± 0.68	27.87 ± 0.53	29.25 ± 0.67	29.50 ± 0.69	28.29 ± 0.64	27.23 ± 0.67
CMS+SYG	100	28.26 ± 0.51	28.50 ± 0.63	29.61 ± 0.68	29.27 ± 0.66	27.14 ± 0.81	27.35 ± 0.78
CMS+SYG	200	27.92 ± 0.42	27.68 ± 0.27	28.67 ± 0.46	28.50 ± 0.32	27.09 ± 0.62	26.67 ± 0.49

Values are expressed as mean ± SEM. ***P* <0.01 compared with Control group.

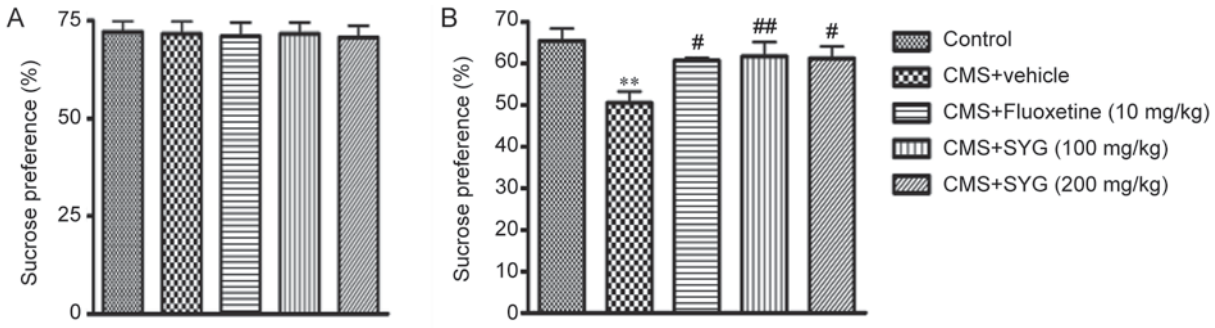

Fig. 3. Baseline sucrose consumption (A) and effect of SYG on sucrose consumption in control and CMS mice (B). Values are the mean ± SEM (*n* = 12). ***P* <0.01 compared with control group, #*P* <0.05, ##*P* <0.01 compared with CMS group.

fluoxetine (10 mg/kg) did not prevent the changes induced by CMS. Besides, no significant difference was detected among groups in the acquisition trial (data not shown).

Effect of SYG on the Concentrations of 5-HT, NE, DA and ACh in the Prefrontal Cortex in Mice Exposed to CMS

The levels of 5-HT, NE, DA and ACh were significantly decreased in the CMS group compared to the control group. Fluoxetine notably increased the 5-HT, but not the DA, NE, and ACh levels *versus* the CMS group. SYG (100 and 200 mg/kg) significantly increased the 5-HT, NE, DA, and ACh levels compared with the CMS group (Fig. 6).

DISCUSSION

In the present study, we investigated the antidepressant effects of SYG on the CMS mouse model, using physical coat state, sucrose-preference, and the novelty-suppressed feeding tests to assess behavior, and measured neurotransmitter levels in the prefrontal cortex. The results demonstrated that SYG treatment at 100 or 200 mg/kg per day had antidepressant-like effects and attenuated the decrease in monoamine levels in the prefrontal cortex of mice exposed to CMS.

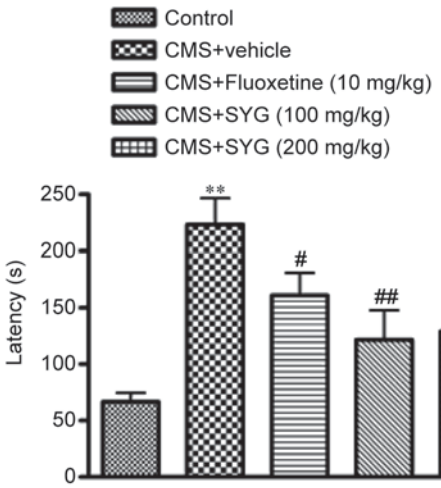

Fig. 4. Effect of SYG on the latency in the novelty-suppressed feeding test in control and CMS mice. Values are mean ± SEM (*n* = 12). ***P* <0.01 compared to control; #*P* <0.05, ##*P* < 0.01 compared with CMS group.

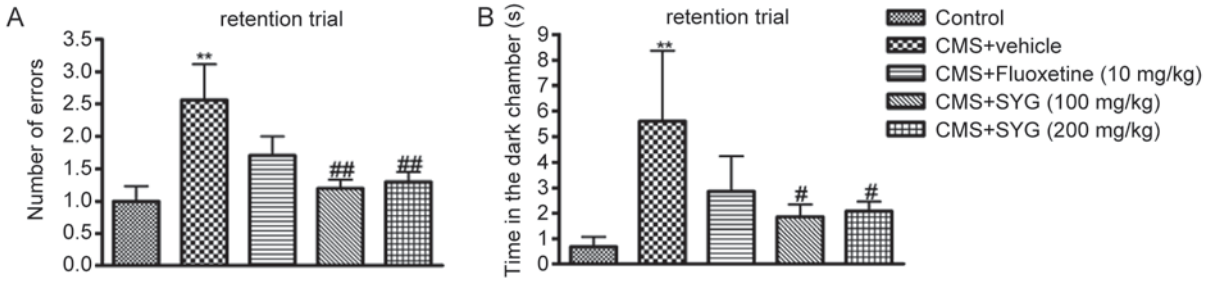

Fig. 5. Effect of SYG on cognition deficit in the step-through passive avoidance test in CMS mice. Values are mean ± SEM (*n* = 12). ***P* <0.01 compared with control group; #*P* <0.05, ##*P* <0.01 compared with CMS group.

It is generally believed that chronic exposure to stress plays an important role in the onset and relapse of depression. Therefore, chronic stress paradigms in laboratory animals constitute important tools in this field. The CMS model, originally developed by Willner[18], has been widely used to study the pathophysiology and therapy of depression because of its combined features of predictive, face, and construct validity[19]. This model induces a variety of behavioral and neurochemical changes resembling those in some depressed patients. CMS-exposed mice exhibit stress-induced degradation in the physical coat state and this effect is reversed by antidepressants such as fluoxetine[20]. In addition, CMS induces anhedonia, a core symptom of depression, which is evaluated by the sucrose-preference test[21]. In the novelty-suppressed feeding test, the latency to feed is elevated by chronic stress, while chronic treatment with antidepressants reverses this effect[22]. Our results showed that CMS induced significant degradation of coat state, reduced sucrose intake in the sucrose-preference test, and increased the latency to feed as compared to control mice, and all of these were reversed by fluoxetine treatment.

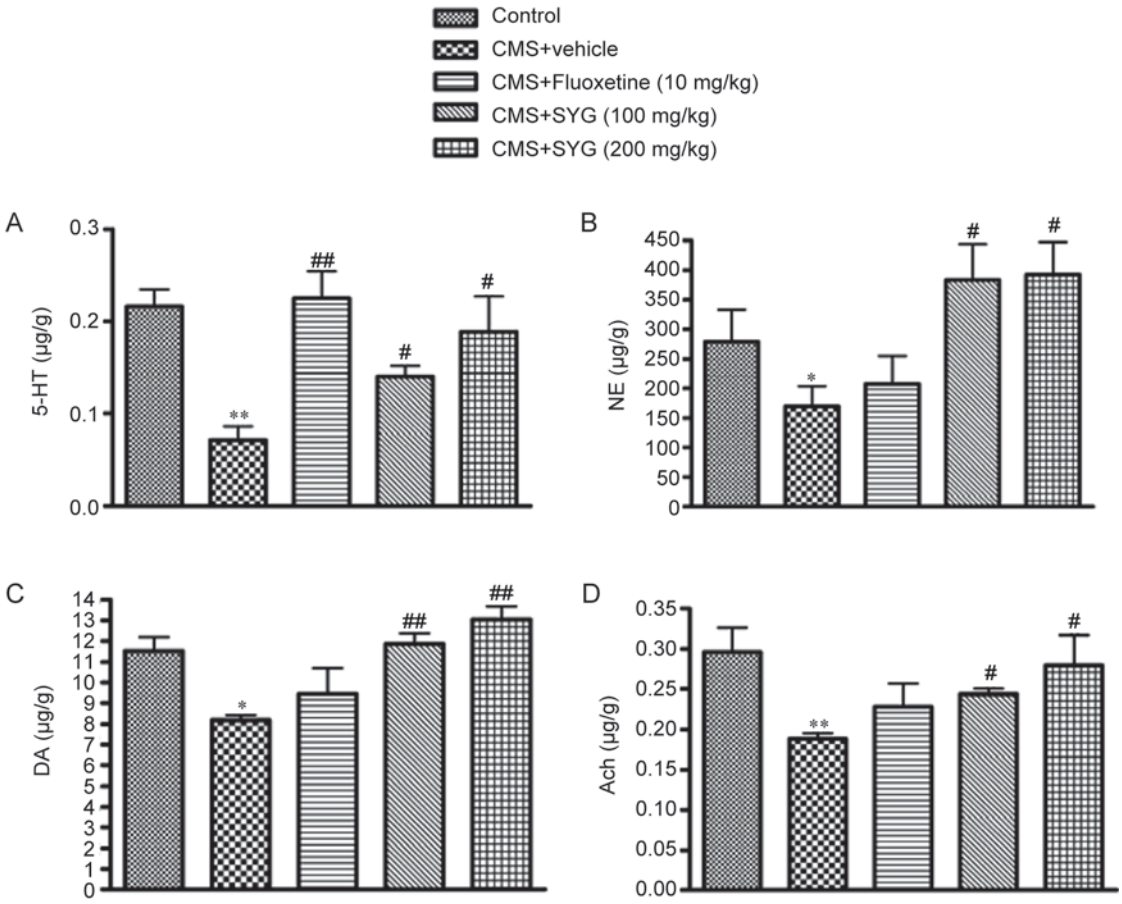

Fig. 6. Effects of SYG and fluoxetine on the 5-hydroxytyptamine (5-HT) (A), norepinephrine (NE) (B), dopamine (DA) (C) and acetylcholine (ACh) (D) levels in the prefrontal cortex of CMS mice. Values are mean ± SEM (*n* = 12). **P* <0.05, ***P* <0.01 compared with control group; #*P* <0.05, ##*P* <0.01 compared with CMS group.

Chronic treatment with SYG (100 and 200 mg/kg) also prevented the changes induced by CMS. These results demonstrated the antidepressant-like effects of SYG in the CMS model.

Although the pathobiology of depression is multifactorial, reduced monoaminergic signaling has long been thought to underlie depressive disorders[23]. The decrement of monoamine neurotransmitter levels (5-HT, DA, and NE) in the prefrontal cortex is thought to contribute to some depressive symptoms[24]. Our results showed that the levels of 5-HT, DA, and NE in the prefrontal cortex were significantly decreased in the CMS model group compared to the control group, which is in line with previous studies using CMS[25]. Chronic treatment with SYG (100 and 200 mg/kg) reversed these changes, which implied that the beneficial action of SYG on the CMS-induced depressive state may be in part based on the prevention of 5-HT, NE, and DA dysfunction in the prefrontal cortex. But it needs to be pointed out that fluoxetine induced a notable increase of 5-HT levels but not those of NE and DA.

Recent studies have shown that cognitive impairment is associated with depression[5]. Some antidepressants have anticholinergic properties that can also lead to clinically significant cognitive impairment such as forgetting, confusion, and concentration problems[26,27]. So it is important to develop new antidepressants with cognitive enhancement. Extracts of *P. ginseng* and *P. tenuifolia* have been used for the treatment of cognitive impairment for many years[28]. Some studies have reported cognitive impairment in stressed animals[29-31]. In our study, a step-through test was performed to evaluate whether SYG ameliorated CMS-induced memory impairment in BALB/c

mice. In the retention trial, mice subjected to CMS showed significantly impaired cognitive performance. SYG at 100 and 200 mg/kg attenuated this impairment, while fluoxetine did not. The cholinergic system is believed to play an important role in cognitive deficits associated with chronic stress[32]. We showed that chronic treatment with SYG (100 and 200 mg/kg) reversed the reduction of ACh levels in the prefrontal cortex, which implied that it might improve cognition by modulating the cholinergic system.

In conclusion, SYG had an antidepressant-like action with memory enhancement in the CMS mouse model. This antidepressant action may be mediated *via* the monoamine neurotransmitter levels in the prefrontal cortex. The cognitive enhancement by SYG may be associated with the ACh augmentation in the prefrontal cortex. Thus, SYG could serve as an alternative medicine for depressed patients with cognitive impairment. This study, to the best of our knowledge, is the first to show that SYG exerts an antidepressant-like action and enhances cognition in the CMS model. However, more research is needed before SYG can be used as an effective antidepressant candidate with cognition-enhancing properties.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Technology of China (2011DFA32730, 2009ZX09103-336), and the S&T Research Project of the PLA, China (BWS11J052).

Received date: 2012-11-08; Accepted date: 2012-12-24

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